THE STRUCTURE AND LOCALIZATION OF GLYCOSPHINGOLIPID ANTIGENS IN THE SMALL INTESTINE AND ALTERATION AT EPITHELIAL CELL DIFFERENTIATION

GUNNAR HANSSON

GÖTEBORG 1981

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GUNNAR C. HANSSON

The present thesis is based on the following publications:

I Breimer, M.E., Falk, K.-E., Hansson, G.C. and Karlsson, K.-A. Structural Identification of two 10-Sugar Branched-Chain Glycosphingolipids of Blood Group H Type in Epithelial Cells of Rat Small Intestine. J. Biol. Chem. in press.

II Ångström, J., Breimer, M.E., Falk, K.-E., Hansson, G.C., Karlsson, K.-A. and Leffler, H. Chemical Characterization of Penta-, Hexa-, Hepta-, Octa- and Nonaglycosylceramides of Rat Small Intestine Having a Globoside-Like Terminal. J. Biol. Chem. in press.

III Breimer, M.E., Hansson, G.C., Karlsson, K.-A. and Leffler, H. Glycosphingolipids and the Differentiation of Intestinal Epithelium. Exp. Cell Res. (1981) 135, 1-13.

IV Breimer, M.E., Hansson, G.C., Karlsson, K.-A. and Leffler, H. Glycosphingolipids of Rat Tissues. Different Composition of Epithelial and Non-Epithelial Cells of Small Intestine. J. Biol. Chem. in press.

ABSTRACT

HANSSON, GUNNAR C., The structure and localization of glycosphingolipid antigens in the small intestine and alteration at epithelial cell differentiation. 1-35. Department of Medical Biochemistry, University of Göteborg, Box 33031, S-400 33 GÖTEBORG, SWEDEN.

The glycosphingolipids are part of the cell surface of mammalian cells. The structure and localization of glycosphingolipids in rat small intestine and their alteration at epithelial cell differentiation have been studied in an attempt to improve the understanding of the functional importance of glycosphingolipids.

Two novel branched blood group H type decaglycosylceramides were isolated from rat small intestine (epithelial cells) and characterized in mixture. The type, number and sequence of sugars were established by degradation of the native mixture and by mass spectrometry and degradations of the permethylated and permethylated-reduced glycosphingolipid mixture. α-L-Fucosidase gave octaglycosylceramides and fucose. Analysis of the permethylated-reduced octa- and decaglycosylceramides by proton NMR spectroscopy showed the anomeric configurations and the localization of type 1 (Galβ1→3GlcNAc) and type 2 (Galβ1→4GlcNAc) sequences on the branches. The structure of the major (60 %) component was

$$\begin{array}{l} \text{Fuc}p\alpha1\rightarrow2\text{Gal}p\beta1\rightarrow3\text{GlcNAc}p\beta1\rightarrow6 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \text{Gal}p\beta1\rightarrow3\text{GlcNAc}p\beta1\rightarrow3\text{Gal}p\beta1\rightarrow4\text{Glc}p\beta1\rightarrow1\text{Cer}. \\ \text{Fuc}p\alpha1\rightarrow2\text{Gal}p\beta1\rightarrow3\text{GlcNAc}p\beta1\rightarrow3 \end{array}$$

The minor (40 %) component had a type 2 chain on the 6-linked branch.

A novel series of glycosphingolipids was isolated from the non-epithelial part of rat small intestine. A mixed fraction containing 3 major components corresponding to glycolipids with 5, 6 and 7 sugars and 2 minor components with 8 and 9 sugars was characterized. The structures of the major components were found to be $\text{GalNAc}p\beta1\rightarrow(3\text{Gal}p\alpha1\rightarrow)_{2\text{-}4}4\text{Gal}p\beta1\rightarrow4\text{Glc}p\beta1\rightarrow1\text{Cer}$. The minor components were concluded, by analogy reasoning, to have one and two additional internal →3Galpα1→3, respectively.

To study changes in lipid-bound cell surface carbohydrates during cell differentiation the epithelial cells of rat small intestine were separated according to maturation (crypt and villus tip cells) as shown by morphological and enzymatic methods. The amount of glucosylceramide in the epithelial cell increased 2-3-fold and N-glycoloylneuraminosyllactosylceramide (hematoside) increased 6-7-fold from crypt to villus tip, but globotriaosyl- and tetrahexosylceramide decreased. No lengthening of the carbohydrate chains was noted, in contradiction to earlier presented ideas concerning cell surface carbohydrates and differentiation. The increased amount of sialic acid in the villus tip cells may facilitate the cell-cell dissociation needed for cell extrusion. There was an increase in 2-hydroxy fatty acids relative to non-hydroxy fatty acids of major glycolipids in cells from crypt compared to villus tip.

The epithelial cells of rat small intestine were separated from their supporting stroma (residue) and the glycosphingolipids in the two compartments were found to be distinctly different. At least 37 different carbohydrate structures were found, 23 in the non-epithelial residue and 17 in the epithelial cells of a white rat strain. Glycosphingolipids with terminal βGalNAc were restricted to the non-epithelial residue comprising globotetraosyl- and isoglobotetraosylceramide in addition to the novel series of glycosphingolipids mentioned above. Fucolipids (blood group H) having 3, 5, 6 and 7 sugars and lacking amino sugars, and fucolipids with 5 and 10 sugars containing N-acetylglucosamine were restricted to the epithelial cells. Blood group type glycosphingolipids and gangliosides based on gangliotetraosylceramide were non-epithelial. In a second rat strain (black-white) another 4 structures with blood group A activity were found in the epithelial cells. This tissue distribution may reflect the different embryonic origin of the epithelial and non-epithelial compartments (endo- and mesoderm, respectively).

Key words: Glycosphingolipids, rat small intestine, epithelial cells, differentiation, tissue localization, blood group antigens.

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Together with M.E. Breimer, K.-A. Karlsson and H. Leffler

These papers will be referred to by their Roman numerals.

Figure on front cover: Molecular model showing the major of the two blood group H type decaglycosylceramides from rat small intestine as seen with the antigenic determinants protruding towards the viewer and the lipophilic chains pointing away into the background. The 6-linked branch is to the right.

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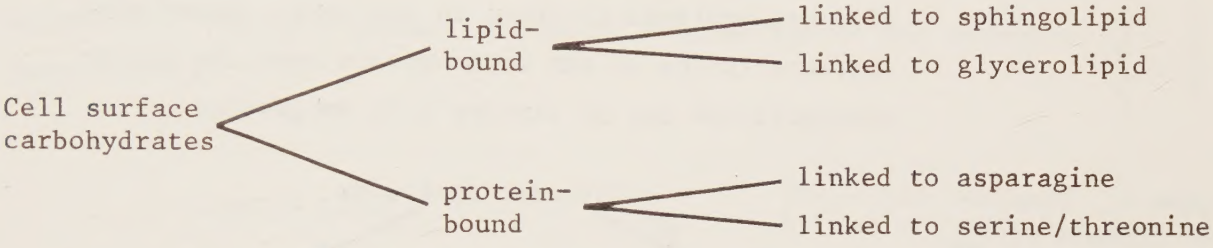
INTRODUCTION

The cell surface of mammalian cells contains membrane-bound carbohydrate [1,2]. This fact and the properties of carbohydrates have raised the hypothesis that the cell surface carbohydrates are involved in various cell surface phenomena including recognition [1]. The immunological recognition of cell surface blood group antigens [1] and the cholera toxin interaction with cells [3] are now well established carbohydrate-dependent cell surface phenomena, whereas the role of carbohydrates in cell-cell interaction is still only poorly understood.

The carbohydrates at the cell surface are linked either to lipids or proteins imbedded in the membrane matrix [1,4].



The cell membrane has a fluid paraffin chain matrix with integral proteins in hydrophobic interactions [5]. The membrane is highly asymmetric [2,6] as shown schematically in Fig. 1. Although most information on these characteristics has been obtained from the erythrocyte it is probable that the cell membrane of cells in the small intestine have similar characteristics [7,8].

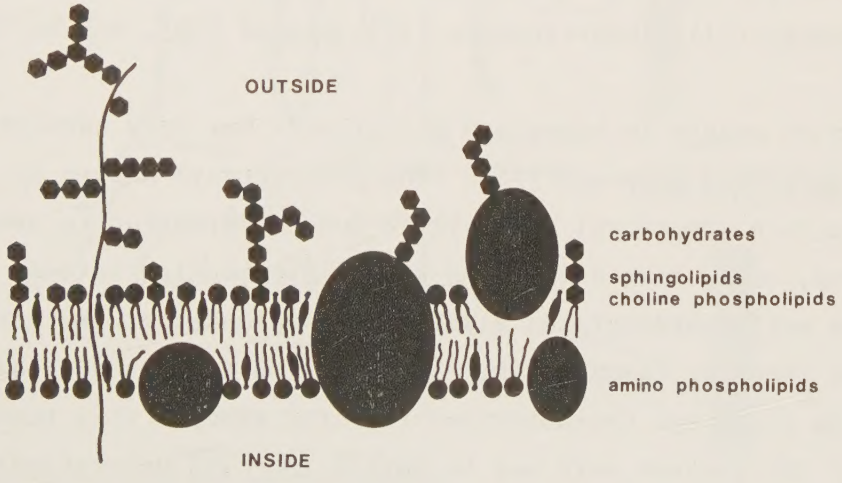


Figure 1. Probable molecular arrangement of the surface membrane of a cell.

GLYCOSPHINGOLIPIDS

Although different types of glycolipids exist the glycosphingolipids comprise the most abundant and most complex group. As this thesis is restricted to the glycosphingolipids the term "glycolipid" has been used as a synonym for glycosphingolipid. A glycosphingolipid is an amphipathic molecule containing a long-chain base with an amide-linked fatty acid (ceramide). The long-chain bases and fatty acids may differ in chain length, double bonds, hydroxylation and methyl branches [9,10].

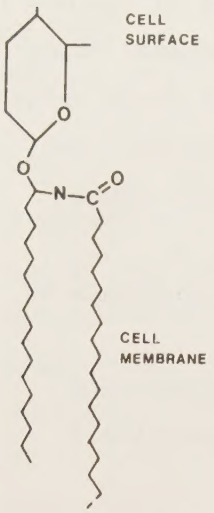
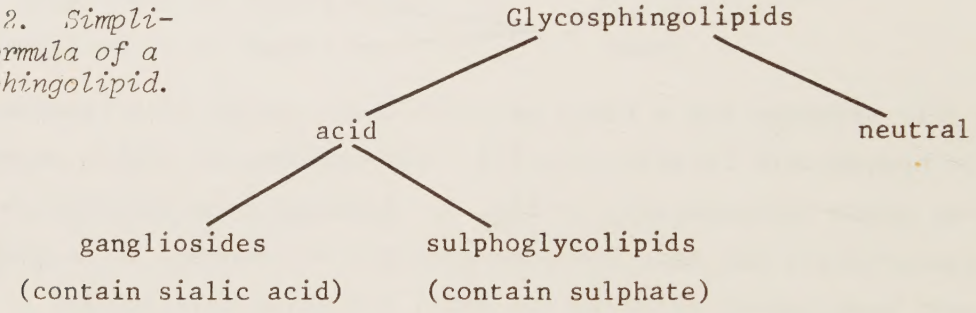


Figure 2. Simplified formula of a glycosphingolipid.

The carbohydrate unit is attached to the primary hydroxyl of the ceramide. A simplified formula of a glycosphingolipid is shown to the left. Based upon characteristics of the carbohydrate part the glycosphingolipids may be divided into subgroups:



Glucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, fucose, N-acetylneuraminic acid and N-glycoloylneuraminic acid are the sugars so far found in mammalian glycosphingolipids, but additional sugars have been found in other vertebrates [11], invertebrates [12], plants [13], and bacteria [14].

The number of sugars in mammalian glycolipids may vary considerably, up to 60 residues have been proposed [15]. The polyhydroxyl nature of carbohydrates gives a rich structural variability due to variation in sequence, binding positions, sugar ring sizes, and glycosidic configurations. About 125 more or less well characterized glycosphingolipid structures have been published so far (see for example [16,17]). Many more structures are probably still to be found and characterized (see for example this thesis).

The aim of the present work was to obtain improved understanding of the structural chemistry of glycosphingolipids in relation to localization and cell differentiation, and of the functional importance of cell surface carbohydrates.

The small intestine was for several reasons considered to be an interesting system for the study of cell surface glycosphingolipids in relation to cell differentiation and tissue localization. The small intestine as experimental system is outlined in Fig. 3. The small intestine was known to be a rich source of glycosphingolipids, especially fucolipids [18]. The epithelial cells lining the intestine are arranged in a single layer to give a large surface (Fig. 3) [19]. The cells migrate from the crypt bottom (dividing, less differentiated cells) to the villus tip with a successive maturation, a cycle of a few days [19]. Cells of different maturity are possible to prepare [20, III]. Complete removal of the epithelial cells also separates two tissue types (epithelial and non-epithelial) having different ontogenic origins (endo- and mesoderm, respectively) [21].

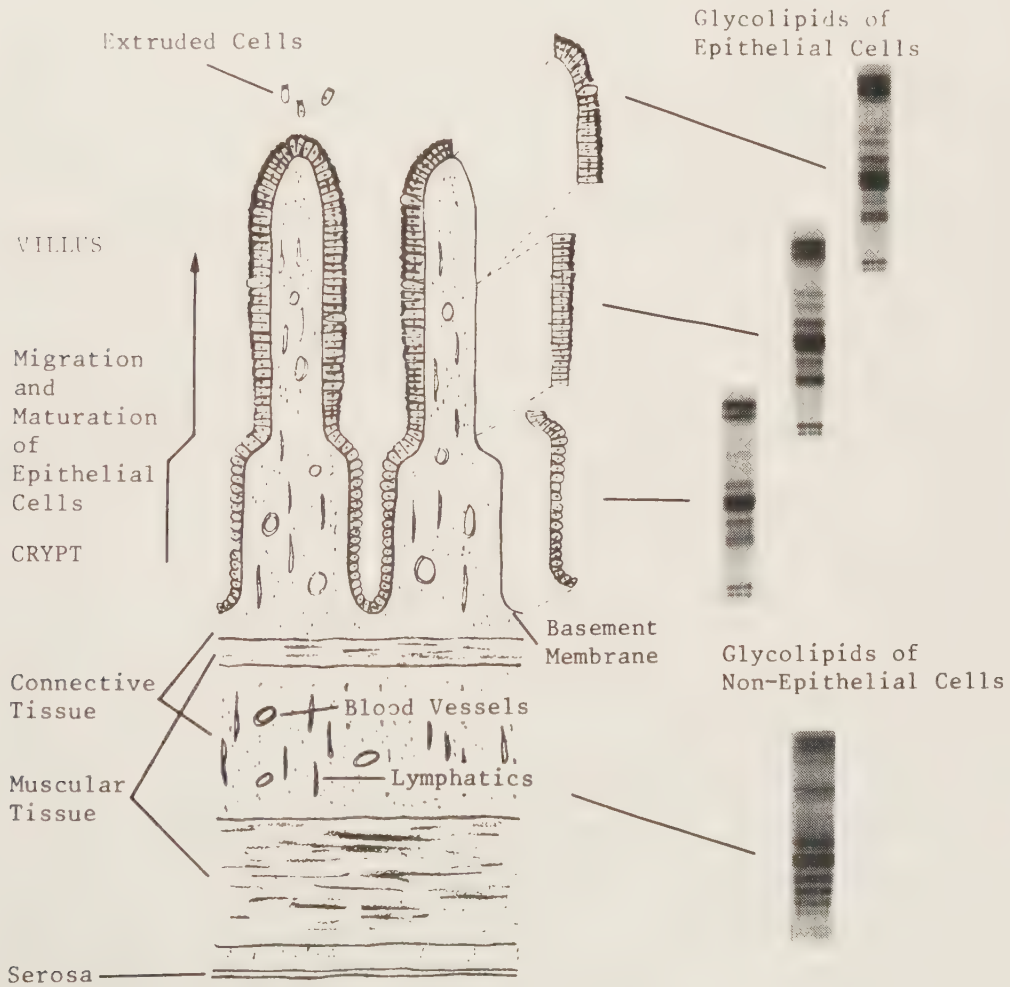


Figure 3. Outline of the morphology of the small intestine showing the experimental system and general approach used in the present study. The single-layered epithelial cells were separated into three fractions according to level of differentiation. The glycolipids of these fractions and of the non-epithelial residue were then analysed using thin-layer chromatography.

To help in selecting an experimental animal and improving our knowledge of species variation the small intestine of 7 different animals including two rat strains was analysed [22-24]. The rat was selected for further studies because of its experimentally suitable size and interesting glycosphingolipid profile. The complexity of glycolipids was large and several novel structures were indicated when subgroups of glycolipids were studied by mass spectrometry [25]. The two rat strains (white and black-white) were also found to have different glycolipid-based blood group antigens, H and A, respectively [26]. Pure glycolipids were prepared and structurally analysed from pooled whole small intestine of the black-white strain [27-32] and from epithelial and non-epithelial cells of the white rat [I,II]. This structural knowledge was then used to characterize alterations during epithelial cell differentiation [III] and determine tissue localization [IV] (see Fig. 3).

METHODS FOR THE CHARACTERIZATION OF GLYCOSPHINGOLIPIDS (I and II)

The isolation of separate glycolipids from a mixture is often difficult and laborious. Several approaches have been used, separately, or in combination [16]: precipitation [33], partitioning [34,35], ion-exchange chromatography [35], silicic acid and Florisil column chromatography of native [35,36], acetylated [25,35,37] or permethylated glycolipids [38], high-pressure liquid chromatography [39] and preparative thin-layer chromatography [18]. The structural characterization of glycolipids with conventional methods, i.e. total or partial enzyme or chemical degradation followed by gas chromatography [16,40-43] needs a pure or almost pure glycolipid. The development of analysis of undegraded glycolipids by mass spectrometry and proton NMR spectroscopy in this laboratory [44-50] has reduced the demand for pure glycolipids [I,II].

The approach for structural characterization of glycosphingolipids as used in papers I and II is outlined in the following and summarized in Fig. 4. Thin-layer chromatography of the native glycolipid gives a rough estimate of the number of sugars and acid degradation the sugar composition [41]. The existence of determinants reactive with antibodies or lectins may be studied by haemagglutination-inhibition [51]. Terminal sugars may be cleaved by specific glycosyl hydrolases [I] and detected together with the remaining glycolipid. The glycolipid is permethylated [52] and $LiAlH_4$-reduced [45] for further studies.

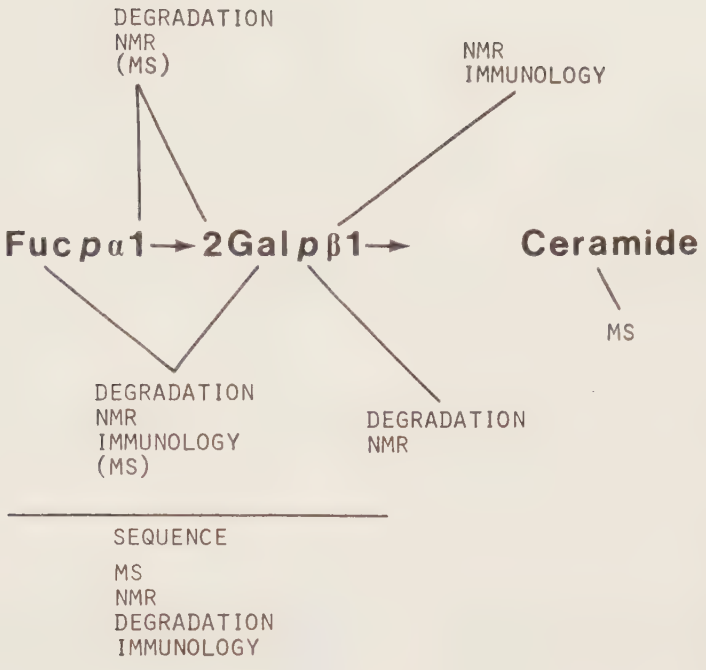


Figure 1. Summary of the information obtained by different methods used for the structural characterization of glycosphingolipids.

Degradation of permethylated and permethylated-reduced glycolipid. The degradation of permethylated glycolipids and gas chromatography (-mass spectrometry) of the partially methylated alditol-acetates give information on binding positions and sugar types [42,43]. Degradation of the permethylated-reduced glycolipid using the same conditions results in the production of di- or trisaccharides containing the amino sugars and their glycosidically bound sugar [46,53]. This acid resistance is probably due to the protonated amine function close to the glycosidic oxygen [46]. Mass spectrometry of the oligosaccharides obtained may give valuable information on linkages and sequences [I,II].

Mass spectrometry. Mass spectrometry has a long tradition at this department and the adaptation of derivatization methods for glycolipids from trimethylsilylation [54,55], acetylation [56] to permethylation, permethylation--reduction [44], and permethylation-reduction-trimethylsilylation [45] has considerably improved the information and extended the achievable mass range. The upper mass limit with the usual technique for introducing the sample into the ion source was recorded at m/e 2533 for a nonaglycosylceramide [46]. Under similar conditions no peaks were obtained at higher mass (Fig. 5) for the branched 10-sugar glycolipids from rat small intestine [I]. However, with a probe technique that introduces the sample

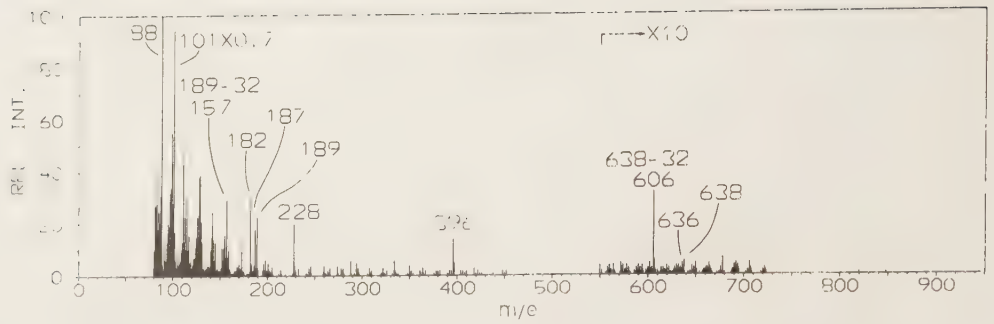


Figure 5. Mass spectrum of the permethylated blood group H type decaglycosylceramides analysed by the conventional technique for introducing the sample into the ion source. Compare Fig. 4 of paper I where the "in-beam technique" was used for the same sample.

very close to the electron beam, "in-beam technique" [57,58], peaks up to m/e 2800 were established from the permethylated-reduced decaglycosylceramides [I].

Mass spectrometry of the two (or three) derivatives of glycolipids provides information on number and sequence of sugars including branching points, on type of sugar (hexose, N-acetylhexosamine, deoxyhexose, N-acetylneuraminic acid or N-glycoloylneuraminic acid), in some instances on linkage positions [46,53, I], and on ceramide components.

Proton NMR spectroscopy. Proton NMR spectroscopy has been used for analysis of oligosaccharides and glycoproteins [59]. The use of permethylated and permethylated-reduced glycolipids dissolved in C^2HCl_3 made also the amphipathic glycolipids accessible to analysis [48-50]. Glycolipids have recently been analysed underivatized in dimethylsulfoxide [60] and ^{13}C NMR spectroscopy may also be used for glycolipids [61]. The NMR method for permethylated and permethylated-reduced glycolipids is very useful as it presents information on anomerity as well as sugar sequence [48-50] and this is applicable also on minor components in a mixture [62,63, I,IV].

TWO BLOOD GROUP H TYPE DECAGLYCOSYLCERAMIDES - EPITHELIAL CELL COMPONENTS (I)

Thin-layer chromatography of the glycolipids in the small intestine from the two rat strains revealed at an early stage of the investigation the presence of one glycolipid band in the 8-10 sugar region in the white rat and one in the 10-12 sugar region in the black-white rat (see Fig. 9). This and the difference in blood group activity between the two strains gave the suspicion that the structures contained two similar determinants each (blood group H and A, respectively) and were thus probably branched. This was settled when mass spectra were provided from non-degraded derivatives

and a trisaccharide was found after degradation of the permethylated--reduced glycolipids. After a careful interpretation of NMR spectra before and after defucosylation the presence of two mixed isomers of the 10-sugar glycolipid was finally established.

Paper I presents the structural data for these two isomers prepared from epithelial cells [IV] and whole small intestine of the white rat strain. The two formulas are shown in Fig. 6 together with molecular models of probable conformations. 60 % of the natural mixture had type 1 sequences at all three positions and 40 % had two type 1 sequences but one type 2 sequence on the 6-linked branch.

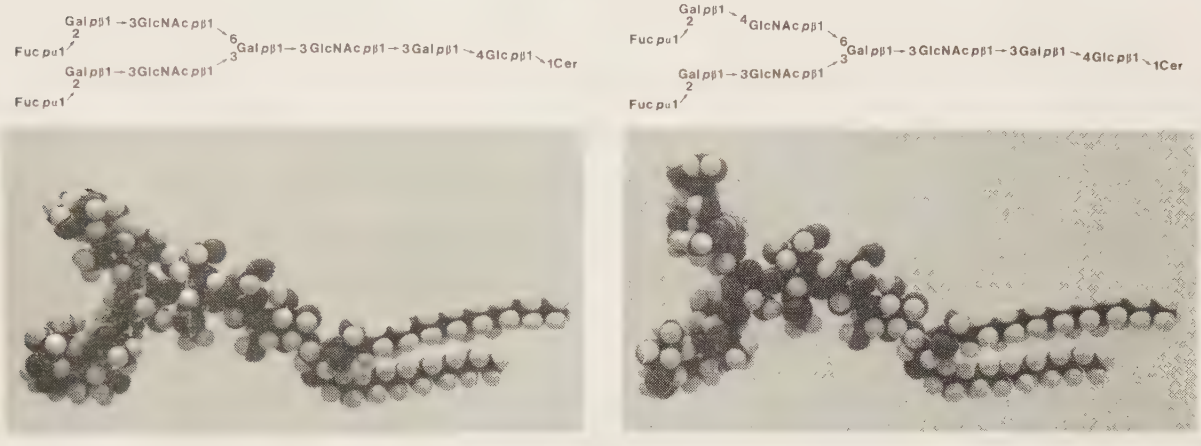


Figure 6. Saccharide formulas and molecular models of the major blood group H type decaglycosylceramides with type 1 chains at all three positions (left) and with one type 2 chain on the 6-linked branch. The conformation of lactosylceramide is similar to that proposed in paper II, that of GlcNAcβ1→3 and the type 2 sequence are from [64], and the other from [65] except the β1→6 linkage that is arbitrary.

A SERIES OF FIVE GLOBOSIDE-LIKE GLYCOSPHINGOLIPIDS - NON-EPITHELIAL COMPONENTS (II)

A series of glycosphingolipids was isolated from the non-epithelial residue of the white rat [IV] and characterized in a mixture. The natural mixture contained 3 major (5, 6 and 7 sugars) and 2 minor components (8 and 9 sugars). The amount obtained of this mixture was too low to permit further subfractionation. The structures of the two minor components were however difficult to establish safely except for the sequence information obtained by mass spectrometry. Their anomerity and binding positions were only tentatively given in analogy with the lower homologues.

This series together with the oligohexosylceramide series lacking the terminal N-acetylglucosamine [31, see IV] comprised a novel type of glycolipid with one and up to five Galα1→3 in a sequence. This sequence

probably has a conformation as a straight ribbon with the sugar rings turned 180° at each glycosidic linkage and placed in the same plane [65,66]. Repeated α1→3 linkages are uncommon in nature, but the conformation of this sequence is probably similar to the type found in cellulose [66]. Fig. 7 shows a schematic formula of the probable conformation of the 9-sugar glycolipid.

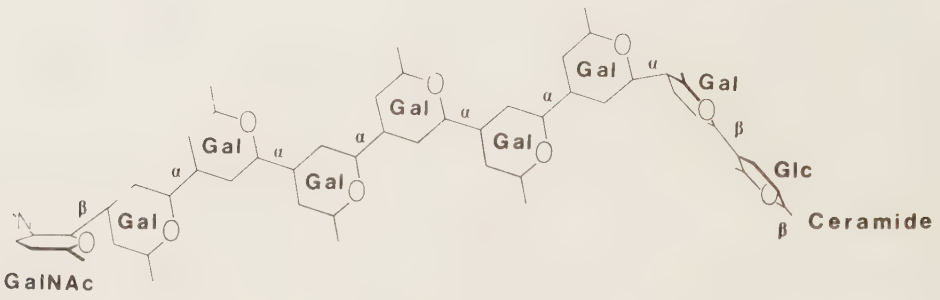


Figure 7. Schematic formula showing the probable conformation of the 9-sugar glycosphingolipid with a globoside-like terminal isolated from non-epithelial residue of rat small intestine.

FRACTIONATION OF EPITHELIAL CELLS (III)

A prerequisite for the studies on glycolipids in cell differentiation and on glycolipid localization was a technique for epithelial cell isolation. This was published [20] but the description was brief and needed adaptation. Briefly described the technique is based on sequential incubations at 37°C with an EDTA-containing buffer and washing out of liberated cells. The cell fractions obtained, separated according to maturity, need to be assayed in each experiment for marker enzymes as the technique is slightly variable [III]. Marker enzymes were selected for immature, dividing cells (thymidine kinase) and mature brush-border bearing villus cells (alkaline phosphatase) [19]. The preparation of total epithelial cells in a larger scale was simplified in the study of tissue localization [IV]. The separation of epithelial cells from the supporting stroma was controlled by microscopy as was the fractional separation of epithelial cells according to maturity [III].

METHODS FOR PREPARATION AND ANALYSIS OF GLYCOSPHINGOLIPID MIXTURES (III,IV)

A prerequisite for the analysis of glycolipids is a general method for the preparation of glycosphingolipids from many sources free of contaminants. Such a method has recently been developed [67]. A simplified and specifically adopted method for the preparation of glycolipids from epithelial cells in a small scale was used in paper III. The extraction was made directly on wet cells using methanol and then chloroform-methanol. After

mild alkaline degradation and dialysis the lipids were separated into non--acid and acid fractions by DEAE-cellulose chromatography. The glycolipids were converted to more lipophilic compounds by acetylation and were finally separated from remaining non-glycolipid substances on silicic acid [37].

The total non-acid glycolipid fraction was analysed by thin-layer chromatography using high-performance silica gel plates (Merck). The detection reagent (anisaldehyde) gives a characteristic green colour for all glycolipids (carbohydrates) with minor differences in hue depending on the constituent sugars [36]. The copper acetate reagent gives a brown colour without discrimination and is better for quantitative purposes and can be combined with densitometry [III].

Mass spectrometry of permethylated and permethylated-reduced total glycosphingolipid mixtures is very useful when combined with a fractional evaporation directly into the ion source. This is obtained with a linearly heated cuvette. The monoglycosylceramides evaporate at 120-200^{o}C while the pentaglycosylceramides evaporate at 240-260^{o}C, giving a partial separation of individual glycolipids. Mass spectra are recorded regularly and stored in a data memory. After the completion of an analysis, curves representing mass versus temperature may be displayed on a computer screen [23, IV]. This method was developed during studies of glycolipids from the small intestine of different animals [22-24]. The method is applicable both for an overview of a glycolipid mixture before a more laborious fractionation and for the presentation of complex results [24,26, IV]. Another approach for glycolipid mixtures giving more information is a fractionation of the acetylated glycolipids into subgroups on one or two silicic acid columns followed by deacetylation and mass spectrometry and proton NMR analysis of the fractions obtained [25,63, IV].

GLYCOSPHINGOLIPIDS AND THE DIFFERENTIATION OF EPITHELIAL CELLS (III)

The philosophy behind this study was the hypothesis of an 'extension response' of cell surface carbohydrates during differentiation. This was included in the model of Roseman [68] with cell surface glycosyltransferases adding single sugars to a carbohydrate back-bone and reformulated by Hakomori [69] in relation to the findings of simpler (shorter) carbohydrate chains in tumour cells. The use of an in situ system in the present study was considered important as most other studies in this field have used cultured cell lines with somewhat contradictory results [70]. According to the model of 'extension response' one would expect to find simpler precursor glycolipids in the less mature crypt cells and extended glycolipids in the villus tip cells.

Soon after the start of these experiments in 1976 a paper by Bouhours and Glickman [71] appeared presenting results on glycolipids made on the same experimental model. These workers had analysed the mono-, di- and trihexosylceramides and the hematoside. We had then detected additional glycolipids and this persuaded us to continue according to our original plans.

The acid and non-acid glycosphingolipids of the crypt and villus tip cells were analysed by thin-layer chromatography and densitometry [III]. This showed that the major alterations were an increase in the amount of glucosylceramide and hematoside and a decrease in tri- and tetrahexosylceramides. This is shown by the total heights of the columns of Fig. 8. The results of the three major components were identical to the results presented earlier [71]. Each mono- to hexaglycosylceramide showed two or

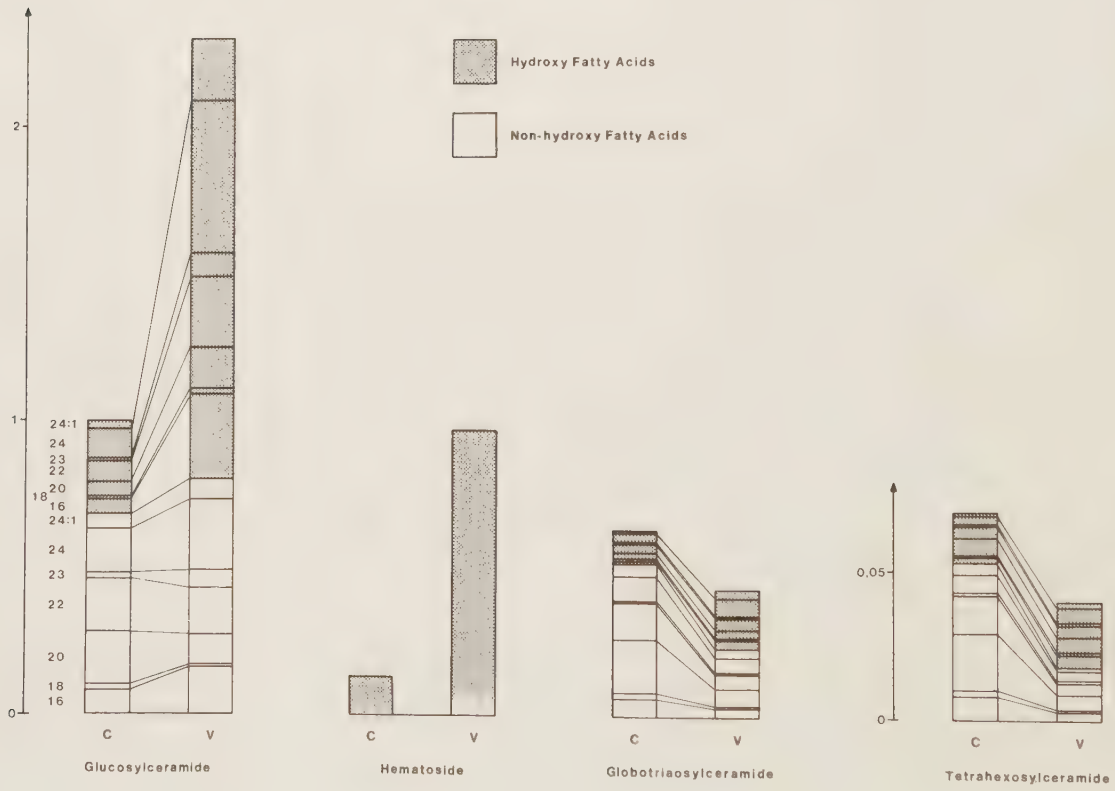


Figure 8. Schematic representation of the amount of the major glycosphingolipids in crypt (C) and villus tip (V) cells of the black-white rat. The fatty acid composition shown is from a separate mass spectrometric study [72]. The amount is expressed in µg glycolipid/mg protein.

three bands on the thin-layer chromatogram [III] that were altered substantially in relative intensity from the crypt to the villus tip. Based on studies of thin-layer mobility [36] and a separate mass spectrometry study of pooled similar fractions [72] it was established that almost all glycolipids contained trihydroxy base [see also IV] and the faster-moving band

mainly non-hydroxy 20,22,24 carbon fatty acids, the intermediate band mainly non-hydroxy 16 and hydroxy 20,22,24, and the slowest-moving band mainly hydroxy 16 carbon fatty acids. The results from [III] and [72] are illustrated in Fig. 8.

No evidence for an 'extension response' was thus obtained except in one case, the hematoside. However, it is not known whether the complex glycolipids found are exposed at the cell surface in both crypt and villus cells or not. Some of the glycolipids might be intracellular until a certain stage of maturation. It may also be a very small cell population within the crypt cell fraction isolated that is "really" undifferentiated. We have probably overlooked other glycolipids if present only in these cells.

THE LOCALIZATION OF THE GLYCOSPHINGOLIPIDS IN THE SMALL INTESTINE (IV)

The tissue localization of glycosphingolipids within the small intestine was analysed in a separate study where epithelial cells and non--epithelial residue from 44 white rats were analysed. Advantage was taken of the knowledge of isolated compounds from whole small intestine [I,II, 27-32,73] and thin-layer chromatography and mass spectrometry (selected ions) of the glycolipid mixtures of epithelial cells and residue from the two rat strains. The background to this study was the knowledge of non-uniform glycolipid localization in the small intestine as revealed by immunofluorescence studies [74,75] and a mucosal scraping [76]. The two compartments are also derived from separate germ-layers during embryogenesis [21].

The glycosphingolipids in the two compartments were distinctly different as shown in Table 1. Most of the structures were characterized separately [27-32,73, I,II], but another 6 were defined in this study.

At least 23 glycosphingolipids were found in the non-epithelial residue and 17 in the epithelial cells of the white rat strain. In the black-white rat strain at least another 4 structures were detected, being blood group A active with 4, 6 and 12 sugars. These were epithelial cell components as were the blood group H active components with or without internal N-acetylglucosamines of the white rat. A blood group B hexa-glycosylceramide and a probable blood group H pentaglycosylceramide were based on gangliotetraosylceramide and non-epithelial as were gangliosides with this core. Glycolipids with four and more sugars having globo and isoglobo terminal disaccharides were non-epithelial.

Table 1. Glycosphingolipids in epithelial and non-epithelial residue of rat small intestine. Human blood group antigens found by immunologic and/or chemical methods are marked.

EPITHELIAL CELLS			NON-EPITHELIAL RESIDUE		
Glycolipid Structures	Blood group[1]	Estimated amount (μg/animal)	Glycolipid Structures	Blood group[1]	Estimated amount (μg/animal)
WHITE RAT			WHITE RAT		
Oligohexosylceramides			Oligohexosylceramides		
Glcβ1 → 1Cer		880	Glcβ1 → 1Cer		170
Galβ1 → 4Glcβ1 → 1Cer		5	Galβ1 → 4Glcβ1 → 1Cer		70
Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer	P^k	410	Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer	P^k	50
Galα1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer		50	Galα1 → 3Galβ1 → 4Glcβ1 → 1Cer		10
Galα1 → 3Galα1 → 3Galα1 → 4Galβ1 → → 4Glcβ1 → 1Cer		< 2	Galα1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer		80
Galα1 → 3Galα1 → 3Galα1 → 3Galα1 → → 4Galβ1 → 4Glcβ1 → 1Cer		< 3	Galα1 → 3Galα1 → 3Galα1 → 4Galβ1 → → 4Glcβ1 → 1Cer		40
Fucolipids			Galα1 → 3Galα1 → 3Galα1 → 3Galα1 → → 4Galβ1 → 4Glcβ1 → 1Cer		25
Fucα1 → 2Galβ1 → 4Glcβ1 → 1Cer	H	25	Galα1 → 3Galα1 → 3Galα1 → 3Galα1 → → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer		20
Fucα1 → 2Galα1 → 3Galα1 → 4Galβ1 → → 4Glcβ1 → 1Cer	H	15	Galα1 → 3Galα1 → 3Galα1 → 3Galα1 → → 3Galα1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer		< 3
Fucα1 → Hex → Hex → Hex → Hex → Hex → Cer		< 2	Fucolipids		
Fucα1 → Hex → Hex → Hex → Hex → Hex → → Hex → Cer		< 3	Galα1 → 3 (Fucα1 → 2) Gal → GalNAc1 → 4Gal → Glc → Cer	B	5
Fucα1 → 2Galβ1 → 3GlcNAcβ1 → 3Galβ1 → → 4Glcβ1 → 1Cer	Le^d	115	With Terminal GalNAc		
Fuc → Hex → HexN → Hex → Hex → Hex → Cer		< 3	GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer	P	110
Fuc → Hex → (HexN ? – Hex) → Hex → Hex → → Hex → Cer		< 4	GalNAcβ1 → 3Galα1 → 3Galβ1 → 4Glcβ1 → 1Cer		210
Fucα1 → 2Galβ1 → 3GlcNAcβ1 → 6 (Fucα1 → 2Galβ1 → 3GlcNAcβ1 → 3) Galβ1 → → 3GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer	Le^d,I	50	GalNAcβ1 → 3Galα1 → 3Galα1 → 4Galβ1 → → 4Glcβ1 → 1Cer		20
Fucα1 → 2Glcβ1 → 4GlcNAcβ1 → 6 (Fucα1 → 2Galβ1 → 3GlcNAcβ1 → 3) Galβ1 → → 3GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer	H,Le^d,I	30	GalNAcβ1 → 3Galα1 → 3Galα1 → 3Galα1 → → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer		20
Miscellaneous			GalNAcβ1 → 3Galα1 → 3Galα1 → 3Galα1 → → 3Galα1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer		< 2
GlcNAc → Hex → Hex → Hex → Cer		< 3	GalNAcβ1 → 3Galα1 → 3Galα1 → 3Galα1 → → 3Galα1 → 3Galα1 → 3Galα1 → 4Galβ1 → → 4Glcβ1 → 1Cer		< 2
			Miscellaneous		
			Galβ1 → 3GalNAcβ1 → 4Galβ1 → 4Glcβ1 → 1Cer		10
BLACK-WHITE RAT			Sialic Acid Containing Glycolipid Structures		
GalNAcα1 → 3 (Fucα1 → 2) Galβ1 → 4Glcβ1 → 1Cer	A	90	NeuAc2 → 3Galβ1 → 4Glcβ1 → 1Cer		180
GalNAcα1 → 3 (Fucα1 → 2) Galβ1 → 3GlcNAcβ1 → 3Galβ1 → → 4Glcβ1 → 1Cer	A	290	NeuGc2 → 3Galβ1 → 4Glcβ1 → 1Cer		25
GalNAcα1 → 3 (Fucα1 → 2) Galβ1 → 3GlcNAcβ1 → 6 Galβ1 → GalNAcα1 → 3 (Fucα1 → 2) Galβ1 → 3GlcNAcβ1 → 3 → 3GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer	A	60	NeuAc2 → 3Galβ1 → 3GalNAcβ1 → 4 Galβ1 → 4Glcβ1 → 1Cer		15
GalNAcα1 → 3 (Fucα1 → 2) Galβ1 → 4GlcNAcβ1 → 6 Galβ1 → GalNAcα1 → 3 (Fucα1 → 2) Galβ1 → 3GlcNAcβ1 → 3 → 3GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer	A	40	NeuAc2 → 3Galβ1 → 3GalNAcβ1 → 3 Galα1 → 3Galβ1 → 4Glcβ1 → 1Cer		30
Sialic Acid Containing Glycolipid Structures			Galβ1 → 3GalNAcβ1 → 4Galβ1 → 4Glcβ1 → 1Cer (3 ↑ 2 NeuGc)		15
NeuGc2 → 3Galβ1 → 4Glcβ1 → 1Cer		640			

[1] Le^d means blood group H type 1 [77]

DISCUSSION

METHODOLOGICAL CONSIDERATIONS

The important prerequisite for successful preparation and characterization of glycosphingolipids is an adequate set of methods. Efficient techniques for the preparation of total glycolipids free of non-glycolipid contaminants are available in this laboratory. [67, III,29]. The combination of preparative methods with mass spectrometry and proton NMR spectroscopy of permethylated and permethylated-reduced glycolipids is highly informative. This is shown in paper IV where the glycolipids were subfractionated as acetylated derivative and analysed by these methods. One of the main difficulties with the conventional degradation methods for structural characterization of glycolipids is the variable recovery of different monosaccharides when the permethylated glycolipid is degraded and analysed by gas--liquid chromatography [43]. This problem is especially apparent for complex glycolipids [78, I], but it can often be surmounted by the mass spectrometry and NMR spectroscopy techniques [I].

The methods of mass spectrometry and NMR can now be further improved by the availability of magnets with higher field strength, thus allowing both improved sensitivity and for the mass spectrometer greater mass range. Other ionization methods such as chemical ionization and field desorption may prove to be useful [79,80], but the new fast atom bombardment (FAB) technique seems to be the most promising at present [81].

The development of the monoclonal antibody technique [82] has presented a most useful tool for the study of glycolipids both in tissues and during preparation. The new technique for detection of glycolipid antigens on thin--layer plates [83] will probably improve the experimental approach considerably.

NOVEL GLYCOSPHINGOLIPIDS

Although about 125 glycolipid structures (different carbohydrate moieties) have so far been proposed, a further 24 novel structures were found in the small intestine of the rat. The structures of 2 of these are presented in paper I, 6 in paper II and 6 in paper IV, the remaining are published elsewhere [27,28,30-32] and are included in Table 1.

The nomenclature of glycosphingolipids is based on the core structure as recommended by an IUPAC-IUB commission [84]. The trivial names for core structures are not sufficient for naming the 13 novel glycolipids with one or several Galα1→3 sugars. This series is based on globotriaosylceramide and galabo may be a suitable name. The glycolipid core structures known and their trivial names are shown in Table 2.

Table 2. Core structures of mammalian glycosphingolipids and their nomenclature [84].

Structure	Trivial name
Gala series	
Galα1→4Galβ1→1Cer	Galabiaosylceramide
Globo series	
Galα1→4Galβ1→4Glcβ1→1Cer	Globotriaosylceramide
GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer	Globotetraosylceramide
Galobo series[1]	
Galα1→3Galα1→4Galβ1→4Glcβ1→1Cer	Galobotetraosylceramide
Galα1→3Galα1→3Galα1→4Galβ1→4Glcβ1→1Cer	Galobopentaosylceramide
Isoglobo series	
Galα1→3Galβ1→4Glcβ1→1Cer	Isoglobotriaosylceramide
GalNAcβ1→3Galα1→3Galβ1→4Glcβ1→1Cer	Isoglobotetraosylceramide
Ganglio series	
GalNAcβ1→4Galβ1→4Glcβ1→1Cer	Gangliotriaosylceramide
Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer	Gangliotetraosylceramide
Isoganglio series[2]	
Galβ1→3GalNAcβ1→3Galβ1→4Glcβ1→1Cer	Isogangliotetraosylceramide
Lacto series	
GlcNAcβ1→3Galβ1→4Glcβ1→1Cer	Lactotriaosylceramide
Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer	Lactotetraosylceramide
Lactoneo series	
Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer	Lactoneotetraosylceramide

1 Proposed in this thesis

2 Proposed from [85]

BLOOD GROUP ANTIGENS

Several of the human blood group systems are known to be specified by free carbohydrate structures or these bound to lipids, proteins or both [86,87]; i.e. the ABH system [87], Lewis system [87,77], the P system [87], and the iI system [88]. Several of these antigens have been found in the rat small intestine as shown in Table 1. Some of the core structures to these rat blood group antigens have not yet been found in man, i.e. the H and A type tri- and tetraglycosylceramides based on lactosylceramide and the H- and B--type glycolipids based on gangliotetraosylceramide.

The erythrocytes of both rat strains were shown to lack blood group A antigens in the glycolipids and probably also in the glycoproteins

(unpublished observation). On the other hand, they do possess blood group B antigens with a probable structure similar to the structures found in the non-epithelial residue. The large intestine of both rat strains probably contains difucosylglycolipids of unknown serological specificity [89].

CELL SURFACE CARBOHYDRATES

Glycoproteins and glycolipids are constituents of the cell surface (see Introduction) and the polyglycosyl subgroups of these have very similar structures [15,86,90,91]. These polyglycosyl compounds are found in the human erythrocyte and they may have from 20 up to 70 sugar residues and are highly branched in the adult cell [15,90,91]. Nothing is known concerning the polyglycosyl compounds in the small intestine. Similarities are also found for terminal saccharides in glycoproteins and glycolipids when the core structures are of intermediate size [86].

However, despite these similarities there are distinct differences; (1) several of the carbohydrate sequences of glycolipids with few sugars have not yet been found on glycoproteins [17,86], (2) the variations seem to be richer in glycolipids, (3) it is easier to obtain antibodies to glycolipids than glycoproteins by heterologous immunization, see discussion in [92] (also including monoclonal antibodies, see discussion in [93]), (4) the carbohydrates of the glycolipids are closer to the membrane, and (5) the glycolipids may move more easily laterally in the membrane. This latter phenomenon is due to the cytoskeleton system of the cell that has no anchoring point on glycolipids. However, glycolipids may interact specifically with proteins [94-96] and these may secondarily control lateral mobility. The closeness of glycolipids to the membrane may be of importance for the function of glycolipids as illustrated by the interaction of the cholera toxin with the ganglioside G_{M1} [97].

GLYCOSPHINGOLIPID VARIATION BETWEEN SPECIES AND INDIVIDUALS

The glycolipids found in the same organ or cells in different animal species show considerable variations [98], as illustrated in Fig. 9 [22].

The individuality and strain variation of glycosphingolipids is also large, illustrated by the human blood group systems [87], the fucolipids and hematosides in different dogs [18,98], strain specificity of blood groups in the rat [26, IV] and strain specificity of gangliosides in mice [99]. Differences also exist for female and male mouse kidney [100].

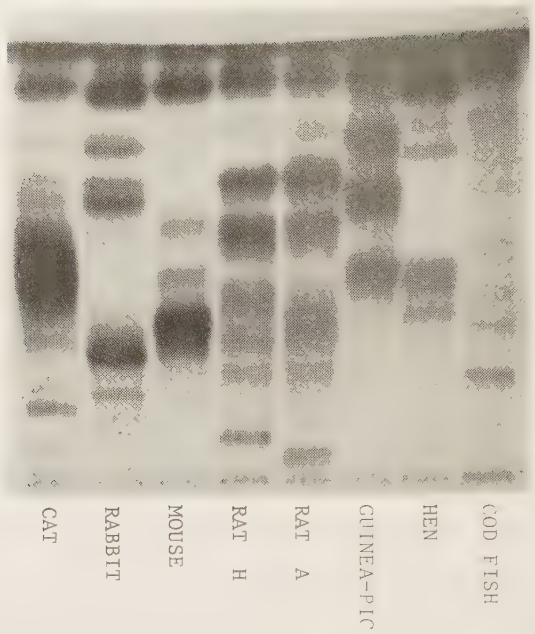


Figure 9. An illustration by thin-layer chromatography of glycosphingolipid differences of small intestine for different animal species and two strains of rat.

TISSUE AND CELL LOCALIZATION OF GLYCOSPHINGOLIPIDS

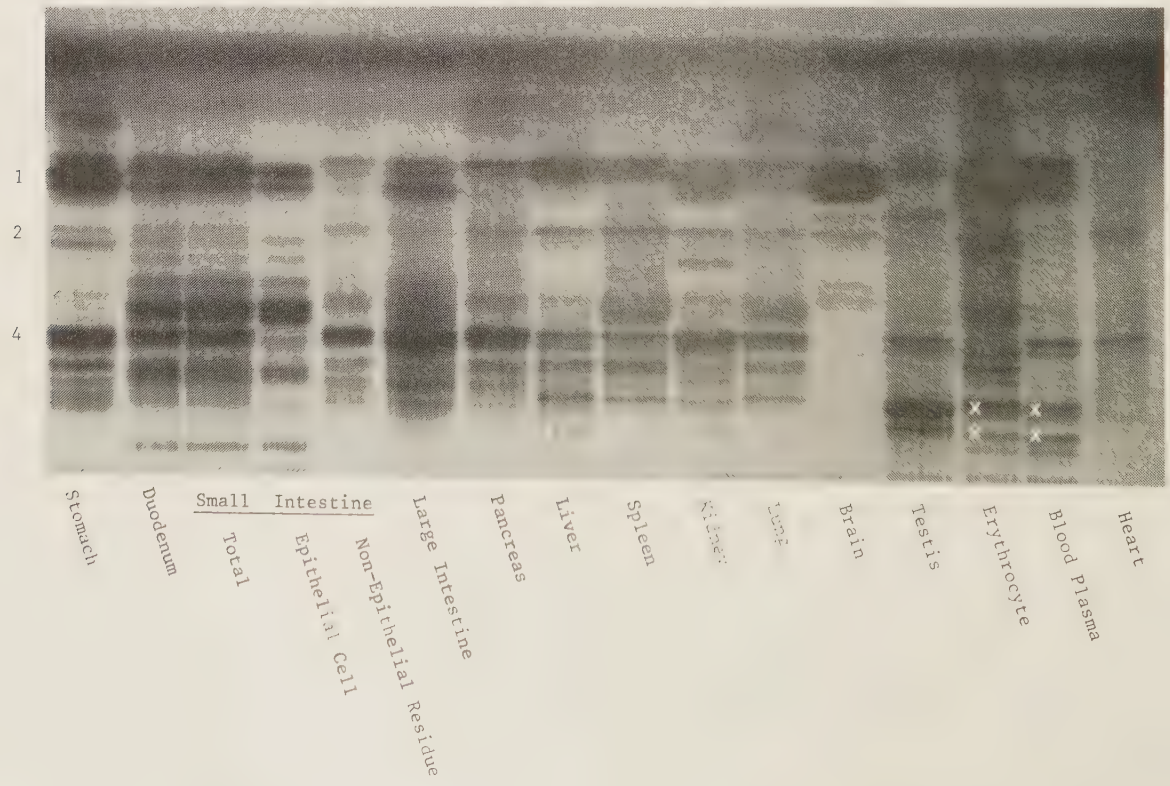


Figure 10. An illustration by thin-layer chromatography of glycosphingolipid differences for different organs within the same rat.
x means non-glycolipid contaminants.

The amount of glycosphingolipids in different tissues varies as does the pattern of glycosphingolipids [4]. The variation of glycolipids in different cells and tissues is illustrated in Fig. 10 [101] showing 14 different organs of the rat.

It is difficult to obtain cells or tissues in sufficient amounts from adult animals that originate from only one germ layer. The epithelial part of epithelial organs also have a secondary support of mesodermal origin [21]. This is also the case for the intestine, but the separation of the epithelial cells from their stroma also separates cells that originate from endo- and mesoderm. Table 3 shows the distribution of terminals and core structures

Table 3. Examples of localization of determinants and core structures found in the small intestine of different animals.

Core Structures	Animals	Small Intestine[1] Epithelial Cells	Non-Epithelial Part
Lactosylceramide	Rat [27,29,30,IV]	+,H,A,NeuGc,(NeuAc)[2]	+,NeuAc,(NeuGc)
	Homo [32,76,102,103]	+,NeuAc	+,NeuAc
Globotetraosyl-ceramide	Rat [IV],Homo [32,76]	-	+
	Cat[3] [22,24]		+,F
	Mouse[3],Guinea-Pig[3],Hen[3] [24],Dog [74,104]		F
Isoglobotetraosyl-ceramide	Rat [IV]	-	+
Gangliotetraosyl-ceramide	Rat [IV,105]	(NeuAc)	+,B,NeuAc,NeuGc
	Mouse [24]	+	
	Homo [32,76,102]	(NeuAc)	NeuAc
Lactotetraosyl-ceramide	Rat [IV,30]	Le^d,A	-
	Homo [32,76,103]	Le^a,Le^b,Le^d,A,B	-
Lactoneotetraosyl-ceramide	Cat[3] [22,24]	A	
	Rabbit[3] [23]	(H or X),A,B,Y	
	Dog[3] [46,104,106,107]	H,A,X,Y	

1. + means that the core structure is present; H,A,B,Le^a and Le^b are the human blood group antigens [87]; Le^d is H on a type 1 chain [77]; F means the Forssman determinant; X means one fucose and Y two fucoses on type 2 chains [77]; NeuAc means N-acetylneuraminic acid and NeuGc N-glycoloylneuraminic acid.
2. The amount of NeuAc in hematoside is large in epithelial cells of duodenum and diminishes in ileum where the NeuGc in hematoside is large [29].
3. These glycolipid localizations were suggested from the type of ceramide present.

within the small intestine of several animals. A general pattern is found, i.e. the globoside and related structures are non-epithelial while all fucolipids based on the two lacto series are epithelial. The ganglio series is not so strictly divided, but the major part is non-epithelial. Although the generality of this distribution pattern in relation to germ layers is difficult to judge for other tissues (Fig. 10) three points may be made: (1) the distribution of globoside and related structures seems to be relatively confined to mesenchymal cells (Table 3 and the immunofluorescence studies of [74,108,109]); (2) the variation of structures present in the epithelial cells of different species is very large (Table 3, Fig. 9); (3) cells and tissues derived from the same germ layer have characteristic glycolipid patterns but the pattern can also be very separate. This last point is illustrated by the presence of a blood group B active tetraglycosylceramide in colon epithelia (absent in small intestine) and the absence in colon of the major H and A glycolipids found in the epithelial cells of small intestine [38]. The non-epithelial part of small and large intestine is very similar [38].

Different glycosphingolipid patterns have also been noticed by same authors [110,111] but not by others [112] within one cell that has two membrane poles as the epithelial cell of small intestine (brush-border and basolateral membranes) divided by a tight junction.

THE SMALL INTESTINE

The intestine is a remarkable organ with a large area exposed to foreign materials including bacteria without the protection of dead cells (compare skin). This kind of barrier must also prevent the digestive enzymes from producing deleterious effects on the epithelial cells and at the same time nutrients must be selectively absorbed. The requirements and the protection of this barrier may be maintained in various ways: a rapid renewal of epithelial cells (2 days) [19], a fast turnover of the brush-border membrane [113] (see below), shedding of brush-border glycoproteins [114], a protective mucus layer [115], protection by the immune system [116] and possibly a protection of the cell membrane by cell surface carbohydrates [117,118]. The crystal structure of the glycoprotein receptor of the influenza virus membrane revealed that the bound saccharide chains (of host origin) lie relatively flat on the protein surface [119]. These studies suggest that the carbohydrate may protect the receptor from antibody attack and degradative enzymes [119,120]. A rapid turnover of the brush-border membrane was also suggested from our studies [72]. From the results in paper III it may be suggested that glucosylceramide and hematoside are synthesized and part of

the tri- and tetraglycosylceramides degraded during the epithelial cell migration from crypt to villus tip. However, by studying the changes of individual fatty acid species in different glycolipids (see ref. 72 and Fig. 8) it was concluded that the turnover rate of individual glycolipids was faster than the entire enterocyte. This has similarly been reported for glycolipids in the rabbit and for glycoproteins in the rat enterocytes [121,113].

The mechanism for the release by EDTA of the epithelial cells from the basal membrane as used in the cell isolation method (III) is not clear. The attachment could however be mediated by fibronectin and/or laminin [122,123]. The cell attachment to collagen mediated by fibronectin is namely EDTA sensitive [124].

SOME FUNCTIONAL CONSIDERATIONS - THE LIPOPHILIC MOIETY

Although no physiological function of the glycosphingolipids has yet been established some functional aspects may be discussed. The lipophilic moiety imbedded in the membrane matrix and the carbohydrate moiety close to the membrane surface often vary in structure independently and are preferably considered separately [10,125].

The sphingolipids are probably restricted to the outer half of the cell membrane where they constitute at least one-third of the lipids [10].

The hydrocarbon chains of the fatty acids and long-chain bases vary in length and saturation for different cell localizations, as do the number of ceramide hydroxyl groups [10]. In the small intestine of the rat this is very characteristic, see Table 4. This apparent relation of

Table 4. Characteristics of the lipophilic moiety in the glycosphingolipids of rat small intestine [III,IV].

	Non-Epithelial Part	Epithelial Cells Crypt	Epithelial Cells Villus Tip
Major chain length of fatty acids	18	20	24
Number of double bonds in long-chain base	1	0	0
Number of hydroxyls (long-chain base + fatty acid)	1 (1+0)	2 (2+0)	3 (2+1)
	→ increased physico-chemical stress on the membrane		

ceramide composition and mechanical and chemical stress on the surface membrane is also present for other tissues [10]. The increased chain length of the glycolipids in the epithelial cells at the villus tip adjusts the membrane fluidity and may add stability to the membrane. The increased number of hydroxyls in the zone between the hydrophobic interior and hydrophilic exterior of the membrane may give more possibilities for lateral hydrogen bonding and thus produce a more stable membrane [10,126].

SOME FUNCTIONAL CONSIDERATIONS - THE CARBOHYDRATE MOIETY

The structural diversity, of sphingolipid-bound carbohydrates at the cell surface, between individuals and very closely related species is rich. The reason for this evolutionary selection towards diversity is not clear. Persons lacking major and 'key' structures in the P- and ABO-blood group systems ("p" lacking globotetraosylceramide and "Bombay" lacking blood group H) are apparently healthy [127]. Most known diseases connected to the glycolipids are due to the absence of degradative enzymes in the lysosomes and an accumulation of glycolipids cause the symptoms [128].

Glycolipids as receptors for pathogenic organisms and toxins. The cholera toxin binds to a glycolipid (a five-sugar ganglioside) [3]. The binding mediates a transfer of the non-binding subunit into the membrane which triggers a series of reactions causing diarrhoea [97]. Other bacterial toxins have also been postulated to have ganglioside receptors, see references in [129].

The attachment of bacteria to epithelial cells is important in the establishment of infections [130]. Different bacterial strains bind to different receptors on the cell surface of which several have been identified in more or less detail, i.e. mannose-containing glycoproteins [131], globotriaosylceramide [132,133], globotetraosylceramide [133], a four-sugar ganglioside [134]. Also, the receptor for bacteria causing diarrhoea in pigs may be a glycolipid [135]. The receptor for Sendai virus has been proposed to be a glycolipid [136].

The fact that different bacterial strains use different receptors may suggest that the bacteria have used many of the potential receptors available on the cell surface. It is interesting that the receptors so far identified are carbohydrates. Since pathogenic organisms and toxins use glycolipids as receptors for invading the host, one may comprehend the usefulness of an individual variation in glycolipids.

Glycolipids as physiological receptors. Gangliosides have been proposed to be receptors for glycoprotein hormones and serotonin, see

references in [129]. The glycolipids are probably not the physiological receptors, but may instead modify or regulate receptor functions [129,137, 138]. Interferon of type I interacts with gangliosides and a ganglioside may act as receptor on the target cell [139,140].

The lymphokine, macrophage migration inhibition factor (MIF) has probably a carbohydrate receptor of blood group type on the macrophage [141, 142]. It was recently found that a blood group B hexaglycosylceramide based on isogangliotetraosylceramide isolated from rat granulomas blocked the binding of MIF to rat macrophages [85]. It thus seems rather plausible that a glycolipid may be the MIF receptor. The finding that MIF contains subunits of which one is binding and the other confers the biological activity [143] may suggest a binding and effector mechanism similar to that of the cholera toxin [97], see above.

A stoichiometric relation between the amount of sulphatide (galactosylceramide-3-sulphate) and the activity of the ion transporting enzyme Na^+-K^+-ATPase has been found for different organs, both with high [144,145] and low transporting capacity [146]. This lipid has a selection of K^+ ion before Na^+ [147] suggesting an involvement of sulphatide in K^+ ion transport [10,148]. On basis of a stereospecific binding to rigid opiates, sulphatide has been proposed to be a part of the opiate receptor [149].

CELL-CELL INTERACTION

Cell-cell interaction is a complex and biochemically a largely unknown process [150]. Carbohydrate structures have been implicated, for example in the interaction of fibroblasts [151], liver cells [152], slime mould cells [153], and the sperm-egg interaction [154]. The sugar moieties of one cell have been proposed to interact with membrane-bound sugars [155], glycosyltransferases [68], lectins [153] or glycosidases [156] of another cell. The presence of cell surface transferases has been questioned [157] and thus the hypothesis of carbohydrate-transferase interaction at the cell surface. The possible involvement of glycosidases in cell adhesion was recently described together with evidence for a cell surface localization of α-mannosidase [124, 156,158].

Carbohydrate binding proteins (lectins) were originally detected in plants, but have since been found in slime mould and animal cells [153]. The lectins of *Dictyostelium discoideum* are developmentally regulated [153], and a mutant defective in one lectin has a blocked cell-cell cohesion at the development from uni- to multicellular organism [159]. The carbohydrate structure recognized by this lectin has not been identified, but a complex glycolipid was altered in a non-aggregating mutant [160]. Some lectins found in animal cells have also been shown to be developmentally regulated [153].

Although no evidence for carbohydrate-protein interaction in cell-cell adhesion of enterocytes exists a lactose-binding lectin has been found in chicken intestine [161] and cell surface glycosyltransferases have been proposed in rat epithelial cells [162]. The increasing amount of N-glycoloyl-neuraminosyllactosylceramide (hematoside) from crypt to villus tip cells [III,71] is parallel to an increased sialylation of endogenous proteins [162]. The charged sialic acid has been shown to block the interaction of glycoproteins with a membrane bound galactose-binding lectin of the liver [163]. The increased amount of sialic acid at the villus tip may help cell-cell dissociation at the villus tip (cell extrusion) and may block the interaction between enterocyte lectin and cell surface carbohydrate. The increased negative charge [164] on the cell surface will also give an increased electrostatic repulsion. Hakomori and coworkers have, in contrast to this hypothesis, proposed the existence of a hematoside-binding membrane protein as a molecular basis of intercellular interaction [165]. However, it is only the glycolipids present in the basolateral membrane of the enterocyte that may be considered involved in cell-cell interaction, whereas the glycolipids of the brush-border may contribute to other types of interactions (for example, with bacteria and toxins).

DEVELOPMENTAL ANTIGENS

The alterations of glycosphingolipids in the epithelial cells of small intestine at maturation was apparently not of qualitative nature [III]. On the other hand, a specific tissue distribution of glycolipids was found [IV] implying that glycolipids may be cell surface markers of mature cells and tissues and possibly also during ontogenic development. The latter statement is substantiated by the different embryonic origins (endoderm and mesoderm) of the two cell populations investigated [IV]. The recent finding of large differences in glycosphingolipids in small intestine of embryos and newly born rats [166] is in agreement with the assumption of glycolipid alterations at development.

Cell surface changes have been found associated with the process of ontogenesis [167,168]. Many of the identified developmental antigens have a carbohydrate nature. Studies with monoclonal antibodies directed against the Forssman glycolipid antigen [169,170] have shown that this antigen is transiently expressed on the trophoectoderm cells and the fetus-forming embryonic ectoderm [171], but persists on the extraembryonic primary endoderm cells. A localization of the Forssman antigen to early mesodermal cells as proposed [IV, 24] has not yet been established [170]. The SSEA-1 (stage-specific embryonal antigen-1) [168], detected by a monoclonal antibody, is a glycolipid with an $\alpha 1 \rightarrow 3$ linked fucose to the N-acetylglucosamine of

of lactoneotetraosylceramide [172], see Table 2. The SSEA-1 has a different cell distribution than the Forssman antigen and is expressed mainly on embryonic ectoderm [168]. Undifferentiated embryonal cells show blood group I activity, that disappears in favour of i at the blastocyst stage [173]. Later I activity is found again in the mouse embryo [173]. Other antigens of glycolipid nature found on preimplantation embryos and teratocarcinoma cells are the I antigen of Ter C cells [174], globoside [175,176] and possibly SSEA-2 [175]. Teratocarcinoma cells can be used as a model of mammalian embryogenesis [171] and the undifferentiated embryonic carcinoma cells synthesize a class of large fucosylglycoproteins, not produced in more differentiated cells [177].

Studies devoted to later stages in development have also shown changes in the cell surface carbohydrate chains. Blood group determinants have been shown to appear and disappear in a certain order during the development of human small intestine [178]. The glycolipid and glycoprotein carbohydrate chains of the human erythrocyte are unbranched until after birth [179]. A conversion to branched type is the basis for the blood group i to I antigen change of the erythrocytes [179]. Other antigens of glycolipid nature are the Thy-1 antigen and gangliotetraosylceramide being markers of lymphocyte development [180,181]. Meconium is a source of fetal antigens giving possibilities to prepare glycolipids of this nature [182].

The studies discussed together with our results indicate that glycosphingolipids may be cell surface markers during ontogenic development. However, further studies are needed to define a more detailed picture as for example the first appearance in mesodermal cells of globoside and related substances. According to the scattered immunohistochemical studies published so far the glycolipid antigens are appearing and disappearing in some cells and maybe reappearing in others during development. This may reflect an addition of extra sugars, as for example proposed for a conversion from I to SSEA-1 antigen during mouse embryogenesis [172] and for gangliotetraosylceramide to ganglioside G_{M1} during lymphocyte development [181,183]. The alterations of cell surface antigens observed may also reflect changes in the cell surface topography [69] with a masking of antigens. This is exemplified by divergent results on the expression of gangliotetraosylceramide on lymphocytes [181,184]. However, it is already now clear that glycolipids detected by immunological methods can define specific stages during embryogenesis (stage-specific antigens) [168], although the functional significance is unknown.

The characteristics of glycolipid antigens and other carbohydrate antigens so far found during ontogenesis is somewhat different from the so-called "lineage" antigens that are more restricted to certain cell lineages and more similar between different species [185]. This has led some investigators to put the carbohydrate-based developmental antigens into a group called "jumping" antigens [185,186] having characteristics for glycolipids as discussed in other parts of this thesis, as for example large species differences for similar organs. These two "systems" of developmental antigens have together been proposed to be a flexible way for the generation of cell-surface diversity [185].

TUMOUR ANTIGENS

Similarities between fetal and tumour antigens are known [187]. Glycosphingolipids have been considered as tumour-associated antigens [92,129] in addition to developmental antigens already discussed.

Two major types of changes have been found in tumour cells compared to normal [129]. (1) Accumulation of simpler and precursor glycolipids not always found in normal cells. (2) Synthesis of new antigens absent (or existing in small amounts) in normal cells. An example of the latter is the appearance of the Forssman hapten in bilary adenocarcinoma, colon cancer and lung cancer of man [188-190]. Recently, a monosialoganglioside was detected as the antigen reacting with a monoclonal antibody obtained by immunizing with colon carcinoma cells [83]. The ganglioside was only found in cancer cells and fetal tissue (meconium).

CONCLUSIONS

The subject of this thesis was the small intestine of the rat. It was shown to have a complex pattern of about 40 separate glycosphingolipids which differed in their localization to epithelial cells and non-epithelial part. Using methods of mass spectrometry and NMR spectroscopy several new structures were identified. These included ten-sugar branched-chain blood group H type glycolipids and a series of structures having up to nine sugars with repeated Galα1$\rightarrow$3 residues. The specificity of glycosphingolipid localization was proposed to reflect the tissue development.

A preparative method for the isolation of epithelial cells of different maturity (cells from the crypt to the villus tip) was improved and used to test current ideas on glycolipid changes upon cell differentiation. No evidence for an 'extension response' was found, that is, a successive growth of saccharide chains with differentiation. However, a seven-fold increase of sialic acid was found, in addition to changes in concentration of some glycolipids and in lipophilic components. The increase of sialic acid was proposed to be of importance for the cell extrusion at the villus tip (less adhesiveness).

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